Toward Solving the Diagnostic Dilemma of Tuberculosis

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Executive Vice President, Chief Medical and Technology Officer - Cepheid
Chief Scientific Officer - Danaher
Consulting Professor, Department of Pathology - Stanford University School of Medicine
1. The Sustainable Development Goals (SDGs) for 2030 were adopted by the United Nations in 2015.

2. One of the targets is to end the global TB epidemic.

3. The WHO End TB Strategy, approved by the World Health Assembly in 2014, calls for a 90% reduction in TB deaths and an 80% reduction in the TB incidence rate by 2030, compared with 2015.

4. This global TB report was the first to be produced in the era of the SDGs and the End TB Strategy.

5. Data were available for 202 countries and territories that account for over 99% of the world’s population and TB cases.
1. Global exposure of TB in 2015: about 1/3 of world population

2. 10.4 million new TB cases in 2015, including 1.2 million cases among people with HIV

3. 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children.

4. Six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South

5. Rate of decline remained at 1.5% from 2014 to 2015.

6. In 2015, there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 people with documented rifampin resistance
<table>
<thead>
<tr>
<th>High-Burden Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afghanistan</td>
</tr>
<tr>
<td>Bangladesh</td>
</tr>
<tr>
<td>Brazil</td>
</tr>
<tr>
<td>Cambodia</td>
</tr>
<tr>
<td>China</td>
</tr>
<tr>
<td>DR Congo</td>
</tr>
<tr>
<td>Ethiopia</td>
</tr>
<tr>
<td>India</td>
</tr>
<tr>
<td>Indonesia</td>
</tr>
<tr>
<td>Kenya</td>
</tr>
<tr>
<td>Mozambique</td>
</tr>
<tr>
<td>Myanmar</td>
</tr>
<tr>
<td>Nigeria</td>
</tr>
<tr>
<td>Pakistan</td>
</tr>
<tr>
<td>Philippines</td>
</tr>
<tr>
<td>Russian Federation</td>
</tr>
<tr>
<td>South Africa</td>
</tr>
<tr>
<td>Thailand</td>
</tr>
<tr>
<td>Uganda</td>
</tr>
<tr>
<td>UR Tanzania</td>
</tr>
<tr>
<td>Viet Nam</td>
</tr>
<tr>
<td>Zimbabwe</td>
</tr>
</tbody>
</table>

**Incidence = rate**

**FIGURE 1**
Estimated TB incidence rates, by country, 2009

[Map showing estimated TB incidence rates per 100,000 population, with color codes for incidence rates ranging from 0-24 to ≥300.]
Pathogenesis of TB – kids are different

- Risk of developing disease after exposure
  - 43% <1 yr
  - 25% age 1-5 yr
- Children with HIV have 6-fold increased mortality
- Often nonspecific presentations
Tests to Diagnose Pulmonary TB Today

1. Sample types for organism detection
   a. Sputum and surrogates (Induced sputum; gastric aspirate; np aspirate; string sample; stool)
   b. Tissue biopsy material

2. Sample types for antigen, antibody, or reactivity detection
   a. Urine
   b. Serum
   c. Whole blood
   d. Breath

3. Types of tests
   a. Smear and culture
   b. Molecular assays
   c. Interferon-G release assays (IGRAs)
Figure 1. QuantiFERON-TB Gold In Tube (QFT-IT) Technology

Part 1. Blood Incubation
- After blood collection, mix QFT tubes thoroughly by shaking vigorously for 5 seconds.
- As soon as possible, and within 16 hours of collection, incubate tubes upright at 37°C for 16–24 hours.
- Incubated tubes are stable for up to 3 days at room temperature, enabling shipment to laboratory.
- Centrifuge tubes at 2000–3000g (RCF) for 15 minutes.

Part 2. IFN-gamma ELISA
- Add 50 μL of working conjugate to each well. Add 50 μL of plasma or standard. Incubate for 120 minutes at room temperature.
- Wash plate 8 times. Add 100 μL of substrate. Incubate 30 minutes at room temperature.
- Add 50 μL of stop solution. Read absorbance at 450 nm (620–650 nm ref).
- Calculate results using QuantiFERON-TB Gold In-Tube Analysis Software, or similar.

- 459 tests: 4.3% indeterminate
- 318 noninfected
- 73 Latent TB Infection
- 68 TB Disease (only 54% had culture confirmation)
- 87% concordance with skin test overall; only 47% in BCG-vaccinated children

### Table 2  Results of the QuantiFERON-TB GOLD In Tube (QTF) test based on final diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBD</td>
<td>61</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>LTBI</td>
<td>32</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>Uninfected</td>
<td>3</td>
<td>304</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>343</td>
<td>20</td>
</tr>
</tbody>
</table>

LTBI, latent tuberculosis infection; TBD, tuberculosis disease.
1. Cavitary disease usually not present; organisms often absent in respiratory secretions (negative smears)

2. Infants and children cannot cough into a container

3. Other sample types (gastric aspirate, induced sputum) hard to obtain

4. Culture yield from intra-thoracic TB = 62%

(Culture-confirmed childhood tuberculosis in Cape Town, South Africa: a review of 596 cases. Schaaf HS, et al. BMC Infect Dis. 2007)
Cavitary TB  

Pediatric TB
Diagnostic approaches for pediatric tuberculosis by use of different specimen types, culture methods, and PCR: a prospective case-control study.


- 218 cases
- 10% positive cultures
### Examples of Molecular Tests for Detection of TB (not all FDA-cleared)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplicor (Roche)</td>
<td>16S rRNA gene; smear +</td>
</tr>
<tr>
<td>MTD (GenProbe)</td>
<td>16S rRNA gene</td>
</tr>
<tr>
<td>Probe-Tec (BD)</td>
<td>16S rRNA &amp; IS6110; smear +</td>
</tr>
<tr>
<td>Xpert Mtb/RIF (Cepheid)</td>
<td>rpoB gene</td>
</tr>
<tr>
<td>LAMP (Eiken; ?Meridian)</td>
<td>gyrA gene</td>
</tr>
<tr>
<td>GTMD (HAIN)</td>
<td>23S rRNA gene; smear +</td>
</tr>
<tr>
<td>Gold nanoparticle probe (Taiwan)</td>
<td>IS6110 &amp; Rv3618</td>
</tr>
</tbody>
</table>

Most still require specimen decontamination & concentration.
Evaluation of reverse transcription loop-mediated isothermal amplification in conjunction with ELISA-hybridization assay for molecular detection of Mycobacterium tuberculosis
Lee et al. 2009. J. Microbiol. Methods 76:174-

Operational Feasibility of Using Loop-Mediated Isothermal Amplification for Diagnosis of Pulmonary Tuberculosis in Microscopy Centers of Developing Countries
Boehme et al. 2007. J. Clin. Microbiol. 1936-

<table>
<thead>
<tr>
<th>Results vs Sputum Culture</th>
<th>SENS</th>
<th>SPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee</td>
<td>94%</td>
<td>83%</td>
</tr>
<tr>
<td>Boehme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear +</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>Smear neg</td>
<td>49%</td>
<td></td>
</tr>
</tbody>
</table>
HAIN GTMD (~5 hr TAT)

Controls

- rpoB mut’s 96% Sens
- katG mut’s 35% Sens
- inhA mut’s 35% Sens
Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillermann, Ph.D., Mark Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Carrilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

Cepheid GeneXpert assay
WHO endorsed

1. Sputum liquefaction and inactivation with 2:1 sample reagent

2. Transfer of 2 ml material into test cartridge

3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)

4. Sample automatically filtered and washed

5. Ultrasonic lysis of filter-captured organisms to release DNA

6. DNA molecules mixed with dry PCR reagents

7. Seminested real-time amplification and detection in integrated reaction tube

8. Printable test result

Time to result, 1 hour 45 minutes
Sensitivity of a single, direct Xpert in S+C+ and S-C+

<table>
<thead>
<tr>
<th>Site</th>
<th>TP</th>
<th>FN</th>
<th>Sensitivity (95 CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lima, Peru</td>
<td>88</td>
<td>0</td>
<td>100 (96-100)</td>
</tr>
<tr>
<td>Baku, Azerbaijan</td>
<td>99</td>
<td>2</td>
<td>98 (93-99)</td>
</tr>
<tr>
<td>Cape Town, SA</td>
<td>49</td>
<td>0</td>
<td>100 (93-100)</td>
</tr>
<tr>
<td>Kampala, Uganda</td>
<td>79</td>
<td>1</td>
<td>99 (93-100)</td>
</tr>
<tr>
<td>Vellore, India</td>
<td>49</td>
<td>0</td>
<td>100 (93-100)</td>
</tr>
<tr>
<td>Manila, Philippines</td>
<td>6</td>
<td>1</td>
<td>86 (49-97)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>370</td>
<td>4</td>
<td><strong>99 (97-100)</strong></td>
</tr>
</tbody>
</table>

**Sensitivity in smear-positive**

**Sensitivity in smear-negative**

Boehme et al. 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study

[www.thelancet.com](http://www.thelancet.com)  April 19
Non-Pulmonary Samples..
Hillemann et al. 2011 JCM 49:1202-5. (FIND Study)

<table>
<thead>
<tr>
<th>Sample</th>
<th>#</th>
<th>Cult +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues</td>
<td>245</td>
<td>30</td>
</tr>
<tr>
<td>CSF</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastric fluid</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>113</td>
<td>0</td>
</tr>
<tr>
<td>Stool</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Urine</td>
<td>91</td>
<td>5</td>
</tr>
</tbody>
</table>

Contaminated cultures = 26
Non-TB mycobacteria = 17

Requires Lab Validation for non-pulmonary samples
Study on TB Prevalence in Cambodian Children

Dr. Rinn Song
Instructor Pediatrics
Harvard Medical School
Currently collaborating with FIND on pediatric TB issues

Dr. Anne Goldfeld
President and Co-Founder, Cambodian Health Committee
Professor of Medicine at Harvard Medical School and Professor of Immunology and Infectious Disease at the Harvard School of Public Health
Study Design

- Clinical criteria assessed (X-ray, skin test, IGRA, etc.)
- Samples collected over 3 days: 2 gastrics; 1 induced sputum; 1 stool
- Gastrics and sputum sent to Pasteur Instit. Lab in Phnom Penh for conventional culture, identification and susceptibilities of isolates (GenProbe; HAIN) and split and sent to Cambodian National TB Lab for Xpert® Mtb/Rif
- Stool frozen for later testing in Xpert® Mtb/Rif
Sample Collection in Cambodia: Aerosol Induction

Albuterol pulse to open airways

15 min breathing saline mist
Aerosol Induction: Step 2
Aerosol Induction: Step 2
Gastric Aspirate
Gastric Aspirate
Gastric Aspirate
Gastric Aspirate
After the Procedures
Enhancing TB Case Detection: Experience in Offering Upfront Xpert MTB/RIF Testing to Pediatric Presumptive TB and DR TB Cases for Early Rapid Diagnosis of Drug Sensitive and Drug Resistant TB

Neeraj Raizada¹, Kuldeep Singh Sachdeva², Sreenivas Achuthan Nair³, Shubhangi Kulsange¹, Radhey Shayam Gupta², Rahul Thakur¹, Malik Parmar³, Christen Gray⁴, Ranjani Ramachandran³, Bhavin Vadera¹, Shobha Ekka¹, Shikha Dhawan², Ameet Babre¹, Mayank Ghedia³, Umesh Alavadi¹, Puneet Dewan³, Mini Khetrapal⁵, Ashwini Khanna⁶, Catharina Boehme⁴, Chinnambedu Nainarappan Paramasivan¹

¹ Foundation for Innovative New Diagnostics, New Delhi, India, ² Central TB Division, Government of India, New Delhi, India, ³ World Health Organization, Country Office for India, New Delhi, India, ⁴ Foundation for Innovative New Diagnostics, Geneva, Switzerland, ⁵ District Tuberculosis Center, Mumbai, India, ⁶ District Tuberculosis Center, New Delhi, India

Accelerating access to quality TB care for paediatric TB suspects in 4 cities of India, though improved diagnostic strategies

Dr. Neeraj Raizada
Project Leader, FIND India
# Xpert MTB/RIF & Smear Microscopy Performance

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Specimen Tested</th>
<th>Xpert Positive (%)</th>
<th>Smear Positive (%)</th>
<th>Rif Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum/IS</td>
<td>10280</td>
<td>769 (7.5%)</td>
<td>334 (3.2%)</td>
<td>86 (11.2%)</td>
</tr>
<tr>
<td>Gastric Asp./Lavage</td>
<td>10026</td>
<td>603 (6.0%)</td>
<td>136 (1.4%)</td>
<td>56 (9.3%)</td>
</tr>
<tr>
<td>CSF</td>
<td>1808</td>
<td>127 (7.0%)</td>
<td>1 (0.1%)</td>
<td>16 (12.6%)</td>
</tr>
<tr>
<td>Pleural Fluid</td>
<td>733</td>
<td>29 (4.0%)</td>
<td>7 (1.0%)</td>
<td>4 (13.8%)</td>
</tr>
<tr>
<td>BAL</td>
<td>647</td>
<td>96 (14.8%)</td>
<td>16 (2.5%)</td>
<td>8 (8.3%)</td>
</tr>
<tr>
<td>Pus</td>
<td>303</td>
<td>123 (40.6%)</td>
<td>29 (9.6%)</td>
<td>11 (8.9%)</td>
</tr>
<tr>
<td>Lymph Node/ FNAC</td>
<td>281</td>
<td>101 (35.9%)</td>
<td>13 (4.6%)</td>
<td>14 (13.9%)</td>
</tr>
<tr>
<td>Ascetic Fluid</td>
<td>149</td>
<td>4 (2.7%)</td>
<td>1 (0.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Others*</td>
<td>272</td>
<td>40 (14.7%)</td>
<td>11 (4.0%)</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24,499</strong></td>
<td><strong>1,892 (7.7%)</strong></td>
<td><strong>548 (2.2%)</strong></td>
<td><strong>202 (10.7%)</strong></td>
</tr>
</tbody>
</table>

*Others* = Tissue, Pericardial Fluid, Urine, Cervical Aspirate, Peritoneal Fluid, Tracheal aspirate, Abscess, Synovial Fluid, Serum Bone, Chyle fluid, Nasal Aspirate, Pleural Biopsy, Thoracic swab

Xpert MTB/Rif not validated for non-respiratory specimen types

Raizada et al.
# Rifampicin Resistant Pediatric Cases

<table>
<thead>
<tr>
<th>Total Suspects</th>
<th>Total Xpert Positives</th>
<th>Total Rif Resistant</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22079</td>
<td>1,735 (7.9%)</td>
<td>156 (9.0%)</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smear Status</th>
<th>Total</th>
<th>Total Xpert Positives</th>
<th>Total Rif Resistant</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>1,057</td>
<td>60 (5.7%)</td>
<td>3 (5.0%)</td>
<td>1.9%</td>
</tr>
<tr>
<td>Smear Negative</td>
<td>20,474</td>
<td>1,142 (5.6%)</td>
<td>94 (8.2%)</td>
<td>60.3%</td>
</tr>
<tr>
<td>Smear Positive</td>
<td>548</td>
<td>533 (97.3%)</td>
<td>59 (11.1%)</td>
<td>37.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Past History of TB Rx</th>
<th>Unknown</th>
<th>1,256</th>
<th>57 (4.5%)</th>
<th>0 (0.0%)</th>
<th>0.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative History</td>
<td>18,253</td>
<td>1,377 (7.5%)</td>
<td>92 (6.7%)</td>
<td>59.0%</td>
</tr>
<tr>
<td></td>
<td>Positive History</td>
<td>811</td>
<td>301 (37.1%)</td>
<td>64 (21.3%)</td>
<td>41.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H/O contact with TB Patient</th>
<th>Positive History</th>
<th>89</th>
<th>57 (63.6%)</th>
<th>0 (0.0%)</th>
<th>0.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative History</td>
<td>50</td>
<td>32 (64.0%)</td>
<td>0 (0.0%)</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>17</td>
<td>10 (58.8%)</td>
<td>0 (0.0%)</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group (in years)</th>
<th>&lt;5</th>
<th>7419</th>
<th>376 (5.1%)</th>
<th>30 (8.0%)</th>
<th>19.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 to 9</td>
<td>7362</td>
<td>405 (5.5%)</td>
<td>39 (9.6%)</td>
<td>25.0%</td>
</tr>
<tr>
<td></td>
<td>10 to 14</td>
<td>7298</td>
<td>954 (13.1%)</td>
<td>87 (9.1%)</td>
<td>55.8%</td>
</tr>
</tbody>
</table>

- 60% of rif resistant cases were smear negative
- 59% of rif resistant cases had no prior history of anti TB treatment
- 32% of rif resistant cases had no history of contact
- Same level of rif resistance observed in all three age groups
- Rif resistance detected in all age grps; Better correlation with H/O contact as compared to H/O past RX; >50% of cases- smear negative
MTB/Rif Ultra: Next Generation Test

- Current test is smear replacement
  - More sensitive than smear, but not as sensitive as culture
- No great reason for culture to be more sensitive than a nested PCR assay
- Multi-copy target provides 10-15 fold boost in sensitivity
- High resolution melt: improve accuracy for drug resistance
- ~30 minutes faster
Early in 2014 our collaborative team met to create a TB test with the goal of being as sensitive as culture: The Xpert MTB Ultra
Xpert Ultra: Increased performance with new fluidics and thermal cycling

New Multicopy target

Fully nested amplification – extra sensitivity.

Enhanced sample processing fluidics.

More rapid and better use of thermal cycling.

Time to result Xpert MTB/RIF = 110 min
Time to positive result Xpert Ultra = 80 min (estimated).
Time to negative result = 66 min (estimated).
Xpert Ultra: Increased sensitivity for TB detection

- Xpert MTB/RIF: Detects TB with a **single copy** target (\textit{rpoB} gene)

- Ultra: Detects two different **multi-copy** targets (\textit{IS6110} & \textit{IS1081})
Xpert MTB/Rif Ultra: PCR Tube Size Matters
Limit of detection (LOD) of Ultra versus Xpert in spiked sputum samples

Assay limit of detection

Ultra: 15.5 CFU/ml
Xpert 113.6 CFU/ml
Dynamic range and semi-quantitation using first rpoB real-time signal (average Ct from 10 replicates).
Rifampin resistance testing by Xpert 98% sensitivity/specificity might not be good enough!

Limited ability to detect mixtures of susceptible and resistant TB

Potential difficulty detecting \( rpoB \) 533 C to G mutations (especially in mixtures) could lead to false susceptible results

Occasional false positive for Rifampin resistance in samples with low bacterial loads due to delay of probe D or E!!!!
4 probes identify rifampin-R mutations in *rpoB* by shifting their Tm away from a wild type reference value.

*rpoB* core region. Any mutation = Rifampin resistance

Probes overlap *rpoB* sequence

A clear change in Tm distinguishes wild type from resistant mutant
A Multicenter Diagnostic Accuracy Study Of The Xpert Ultra For Tuberculosis Diagnosis

Presenter: David Alland, MD.

Authors: Samuel G Schumacher¹, Pamela Nabeta¹, Catharina C Boehme¹, Jerrold Ellner², David Alland³, Susan E Dorman⁴, Claudia M Denkinger¹, for the TB Clinical Diagnostics Research Consortium and FIND Trial Consortium

Affiliations: ¹FIND, Geneva, Switzerland, ²Boston Medical Center, Boston, MA, ³Division of Infectious Diseases, Rutgers-New Jersey Medical School, Newark, ⁴Johns Hopkins University, Baltimore, MD
Study Design: Multicenter - 10 sites in 8 countries

- Non-inferiority: Ultra versus Xpert
  - Reference standard culture/DST (4x)
  - Primary endpoint: Δ in sensitivity and specificity between Xpert Ultra and Xpert for detection of MTB and RIF
- Both assays performed on same specimen
- Enrollment
  - Case detection group: patients under evaluation for TB (no TB treatment in past 6 months)
  - MDR risk group: patients under evaluation for TB/MDR-TB (may already be on TB treatment)
- Analyses
  - MTB detection analysis: limited to case detection group
  - RIF detection analysis: done in all participants (Case detection group & MDR risk group)
• Total 1,520 participants met eligibility criteria Feb – Oct 2016
  • 1,243 participants in ‘Case Detection Group’
  • 277 participants in ‘MDR-risk Group’
• Case Detection Group
  • 403 (32.4%) were culture-positive - 119 (29.5%) were smear-negative
  • 840 (67.6%) were culture-negative – ie not TB
• Among all 1,520 participants
  • 187 (12.3%) were rifampin-resistant
  • 416 (27.4%) were rifampin-sensitive
• 25% were HIV-infected and 21% had a history of prior TB
Results: Non-inferiority analysis

Δ sensitivity for HIV-infected: +12% (95%CI +4.9, +21)
Conclusions- MTB/Rif Ultra Studies

- Ultra has superior sensitivity compared to Xpert in smear-negative (+17%) and HIV-infected patients (+12%)
- Ultra also detects TB DNA in some patients with prior TB disease, possibly due to persistence of non-viable bacilli, leading to reduced specificity.
- Improved Rif R accuracy
- Whether *M. tuberculosis* culture-negative but Ultra test-positive patients represent a high risk group for relapse remains to be determined.
- Despite these questions, WHO endorsed Ultra on March 24, 2017.
MTB/RIF Ultra: More Sensitive for Extrapulmonary TB

- TB meningitis is a life-threatening condition and difficult to diagnose
- 128 HIV infected adults tested in Mbarara, Uganda
- Sensitivity of culture: 43% for clinically and microbiologically-proven definite TB meningitis
- Sensitivity of G4: 43% (9/21; P=0.002)
- Sensitivity of Ultra: 95% (20/21)

Quote from David Boulware, MD (PI): “This is a game changer”
After Theranos: What?

After Theranos

The implosion of blood diagnostics developer Theranos has raised the question: What is feasibly detectible in a drop of blood? Emily Waltz reports.

instruments, leading to errors, the newspaper reported. The next month, the CMS inspected Theranos' laboratory in Newark, California, and found numerous deficiencies, some of which posed "immediate jeopardy to patient health and safety," CMS said in a letter to the company (Box 1).

The agency banned Holmes from running a
Blood-Based Tuberculosis Biomarkers

Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis

Lancet Respir Med 2016; 4: 213-224

Timothy E Sweeney, Lindsay Braviak, Cristina M Tato, Purvesh Khatri

- 14 data sets, 2572 samples from 10 countries, adult and pediatric patients
- Only whole blood data included
- 266 genes (158 over-expressed; 108 under-expressed)
- Narrowed down to 3 genes
- Robert Wallis, now with Aurum Institute, already developing a test

No effect of HIV status
Violin Plots of Different Data Sets (need different cutoff scores)

3 most informative genes from peripheral blood: (DUSP3, GBP5, KLF2)
Validation ROCs

ATB Diagnosis vs healthy, LTB and other diseases
sensitivity = 86%; specificity = 86%; NPV = 99% @ 10% prevalence

Other Important Findings

- Not confounded by HIV co-infection
- May allow monitoring treatment response
- Not confounded by BCG vaccination

Real Data: 3-Gene Signature Maintains Accuracy in Active Case Finding Screen

- Prospectively enrolled in Brazil (Julio Croda and Jason Andrews)
- Active case-finding (low severity patients)
- PCR and culture-defined positivity
Persisting positron emission tomography lesion activity and *Mycobacterium tuberculosis* mRNA after tuberculosis cure

Stephanus T Malherbe, Shubhada Shenai, Katharina Ronacher, Andre G Loxton, Gregory Dolganov, Magdalena Kriel, Tran Van, Ray Y Chen, James Warwick, Laura E Via, Taeksun Song, Myungsun Lee, Gary Schoolnik, Gerard Tromp, David Alland, Clifton E Barry III, Jill Winter, Gerhard Walzl, the Catalysis TB–Biomarker Consortium

Nature Medicine 2016

Active TB diagnosis

Predicting treatment response

Unpublished data

Distinguishes ATB with same accuracy as predicted

Not cured vs. other classes @ 168 days

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**Graphs:**

- **Left graph:**
  - Healthy Controls: AUC=0.94 (95% CI 0.96 – 0.92)
  - Lung Dx Controls: AUC=0.9 (95% CI 0.93 – 0.86)
  - MTP Controls: AUC=0.94 (95% CI 0.96 – 0.92)

- **Right graph:**
  - Definite Cure: AUC=0.92 (95% CI 0.99 – 0.85)
  - Probable Cure: AUC=1 (95% CI 1 – 1)
  - Possible Cure: AUC=0.9 (95% CI 0.99 – 0.82)
  - Unevaluable: AUC=0.96 (95% CI 1 – 0.9)
  - Def Prob Poss: AUC=0.93 (95% CI 0.99 – 0.86)
Summary

- 3-gene whole blood signature
  1. Seems able to distinguish ATB from LTB, other lung diseases and healthy controls
  2. Preliminary but successful validated using PCR in a prospective cohort for active case finding
  3. Can identify treatment non-responders at the end-of-treatment
After Theranos: What?

Potentially a lot:

- HIV qual for case detection and EID
- HIV quant (Gates project)
- Ebola (Gates funded)
- HCV quant
- Active TB?
- Viral versus bacterial
Addressing the Dx Dilemma of TB

• Still very challenging given its nonspecific presentation and paucibacillary nature
• More sensitive detection methods may help
• Validation of non-pulmonary samples, including stool, may help
• Non invasive blood based signatures may hold promise to fill some of the gaps